Review

Interspecific and intergeneric hybridization and chromosomal engineering of Brassicaceae crops

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In Brassicaceae crop breeding programs, wild relatives have been evaluated as genetic resources to develop new cultivars with biotic and abiotic stress resistance. This has become necessary because of the diversification of ecotypes of diseases and pests, changing food preferences, advances in production technology, the use of new approaches such as *in vitro* breeding programs, and the need for economical production of F_1 seed. To produce potential new cultivars, interspecific and intergeneric hybridizations have been performed between cultivated species and between cultivated species and their wild relatives. Furthermore, interspecific and intergeneric hybrids have been successfully produced using embryo rescue techniques. In this paper, we review the interspecific and intergeneric incompatibilities between Brassicaceae crops and their wild relatives, and the production, characterization, and improvement of synthetic amphidiploid lines, alien gene introgression lines, alloplasmic lines, monosomic alien chromosome addition lines, and monosomic alien chromosome substitution lines. The goal is to provide useful materials to support practical breeding strategies and to study the genetic effects of individual chromosomes on plant traits, the number of genes that control a trait, their linkage relationships, and genetic improvement in Brassicaceae crops.

Key Words: interspecific and intergeneric hybridization, chromosomal engineering, Brassicaceae, amphidiploid, monosomic alien chromosome addition, alloplasmic, alien gene introgression.

Introduction

The Brassicaceae comprise about 330 genera and 3700 species, of which two genera (*Brassica* and *Raphanus*) are widely grown for edible oils, vegetables, spices, ornamental flowers, and forage crops around the world. The genus *Brassica* contains three monogenomic diploid species, namely *B. rapa* L. (2n = 20, AA genome), *B. nigra* (L.) Koch (2n = 16, BB), and *B. oleracea* L. (2n = 18, CC), and their naturally produced allotetraploid species, *B. napus* L. (2n = 38, AACC), *B. juncea* (L.) Czern. & Coss. (2n = 36, AABB), and *B. carinata* A. Braun (2n = 34, BBCC). In contrast, the genus *Raphanus* contains only one species of agricultural importance, *R. sativus* (2n = 18, RR) (Mizushima 1950). Other crop species in this family include *Eruca sativa* L. (rocket salad), *Nasturtium officinale* R. Br. (watercress), *Wasabia japonica* Matsumura (wasabi), *Matthiola incana* (L.) R. Br. (stock), and *Erysimum cheiri* (L.) Crantz (English wallflower). A number of wild relatives, on the other hand, have been evaluated as genetic resources in the development of new cultivars with biotic and abiotic stress

resistance for use in agriculture in response to the diversification of ecotypes of diseases and pests, changing food preferences, adoption of advanced production technology, the use of new approaches such as *in vitro* breeding programs, and the need for economical production of F_1 seed (Warwick 1993).

Since *Raphanobrassica*, an intergeneric hybrid between *R. sativus* and *B. oleracea*, was reported by Karpechenko (1928), a number of intergeneric and interspecific hybridizations between *Brassica* species of the U triangle (U 1935) and *R. sativus* have been produced; the U triangle refers to the ancestral A, B, and C genomes that have given rise to modern *Brassica* crop species. For nearly a century, cytogenetic research based on their hybridization has been conducted, resulting in the discovery of excellent evidence for the cytogenetics and speciation of these species. Recently, interspecific and intergeneric hybridizations have been extensively performed between the cultivated species and between cultivated species and wild relatives to develop more potentially useful cultivars with improved biotic and abiotic stress tolerance (Harberd and McArthur 1980, Prakash *et al.* 1999).

However, cross-incompatibility in the interspecific and intergeneric hybridizations has hampered the development of these hybrids. Using knowledge gained from anatomical
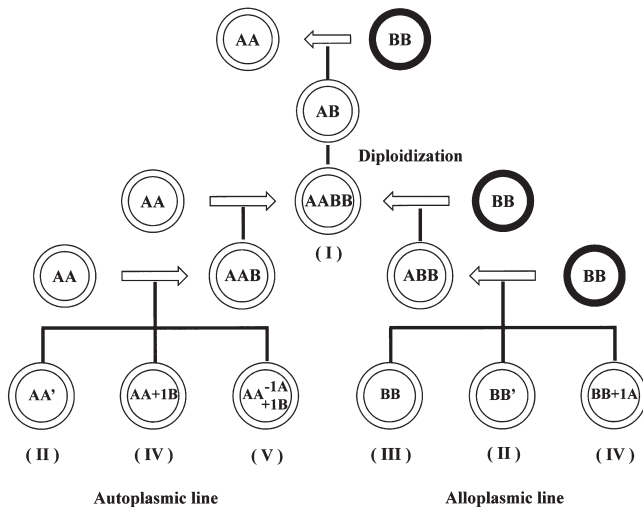
Fig. 1. Schematic diagram of the distant hybridization breeding system between AA and BB genome species concerned (quoted from Matsuzawa *et al.* 1996, revised). (I): Synthetic amphidiploid line (SADL), (II): Alien gene(s) introgression line (AGIL), (III): Alloplasmic line (ALPL), (IV): Monosomic alien chromosome addition line (MAAL), (V): Monosomic alien chromosome substitution line (MASL) 1) AB, AABB and AAB (ABB) show genomes for amphihaploid, amphidiploid and sesquidiploid, respectively. 2) A' (B') means some genetic modification via recombination between each complement of A and B genome. 3) AA + 1B(BB + 1A) means A (B) genome species added single chromosome of B (A) genome species. 4) ○ and ● show the difference in cytoplasmic background for A and B genome species, respectively. 5) A←B means the hybridization in which A and B genome species are pistillate and pollen parents, respectively. 6) In (V), a chromosome of A-genome is substituted by one of B-genome.

studies of the growth of hybrid embryos, Nishi *et al.* (1970) and Inomata (1977) produced interspecific and intergeneric hybrids through embryo and ovary culture techniques, respectively.

Matsuzawa *et al.* (1996) suggested a system for the use of interspecific and intergeneric hybridizations to develop five types of hybrid lines: synthetic amphidiploid lines, alien gene introgression lines, alloplasmic lines, monosomic alien chromosome addition lines, and monosomic alien chromosome substitution lines (Fig. 1). These hybrid lines would be valuable genetic resources both for breeding more productive cultivars with novel agronomic traits and for research to better understand each chromosome and gene in these hybrids. In each instance, it is first necessary to develop true F₁ hybrids and as many of their progeny as possible.

In this communication, we review current knowledge of the interspecific and intergeneric cross-incompatibility between *Brassica* crops and their wild relatives, and the production, characterization, and improvement of the five types of hybrid lines.

1. Hybridization barriers in interspecific and intergeneric hybridizations

The barriers to interspecific and intergeneric hybridization during sexual reproduction can be divided between those that operate before and after fertilization. Stebbins (1958) suggested that the pre-fertilization barriers might be due to failure of pollen germination, pollen tube growth, or pollen tube penetration of the ovule, whereas post-fertilization barriers would arise from degeneration of the hybrid embryo, male and female sterility in the hybrid plants, and lethality in the hybrid progeny. Khush and Brar (1992) surveyed the hybridization barriers in distant hybridization, and offered effective techniques to overcome both pre- and post-fertilization barriers.

Several researchers have investigated the nature of the pre-fertilization barriers for interspecific and intergeneric hybridizations in the Brassicaceae. For example, Matsuzawa (1983) evaluated the magnitude of the pre-fertilization barriers using a pollen germination index:

$$P.G.I. = (1b + 2c + 3d + 4e)/(a + b + c + d + e)$$

where $0 \leq P.G.I. \leq 4$, and a, b, c, d, and e represent the numbers of pistils in which no pollen grain is recognized on the stigma (with a score of 0), pollen grains do not germinate on the stigma (1), pollen grains germinate on the stigma but do not enter (2), pollen tubes reach the style tissue (3), and pollen tubes penetrate the style tissue to reach near or to the ovule (4), respectively. Kerlan *et al.* (1992) proposed a different equation for the index of pollination compatibility (I):

$$I = x + 2y + 3z$$

where x, y, and z are the corresponding scores for the numbers of pollen grains that germinate divided by the number that arrived on the stigma (with a score of 0, 1, 2 or 3 assigned to the ratio values of 0, 0–0.5, 0.5–0.7 and 0.7–1, respectively), of pollen tubes that grew into the style tissue (with a score of 0, 1 and 2 corresponding to 0, 1 to 5 and more than 6 pollen tubes, respectively), and of pollen tubes that penetrated the ovule (with a score of 0, 1 and 2 assigned to the same scores used for the y parameter), respectively.

In general, the pre-fertilization barriers between cultivated species and their wild relatives in interspecific and intergeneric hybridizations can be overcome by means of bud pollination and using wild species as the pistillate parent, although the proportion of successful outcomes may depend on the direction and combination in each crossing. In particular, when a self-compatible line was used as the pistillate parent, the pollen tubes of cultivated species grew well and penetrated into the ovule. Therefore, the pre-fertilization barriers in interspecific and intergeneric hybridizations seem to be similar to the self-incompatibility observed in Brassicaceae species, although genetic analysis using the F₂ population between an interspecific-incompatible line and a self-compatible cultivar could not confirm the responsibility of the line and the cultivar in the differences

of the interspecific incompatibility phenotypes (Udagawa *et al.* 2010).

In contrast to pre-fertilization barriers, Wilmar and Hellendoorn (1968) evaluated post-fertilization barriers by means of an anatomical survey of the development and growth of embryos of Brussels sprouts (*B. oleracea*). The abortion of hybrid embryos in interspecific and intergeneric hybridizations resulted from abnormal development of the endosperm, and the underlying mechanisms have been explained by various hypotheses: the endosperm balance number (Johnston *et al.* 1980), activation of the polar nuclei (Nishiyama and Yabuno 1978), and genomic imprinting in the endosperm (Kinoshita 2007). Recently, Tonosaki *et al.* (2013) performed quantitative trait locus analysis during the formation of hybrid seed in intergeneric hybridization between *B. rapa* and *R. sativus*. Researchers have developed effective *in vitro* culture techniques using pollinated flowers, ovaries, ovules, and embryos to rescue hybrid embryos that would otherwise degenerate during the early stages of their development in interspecific and intergeneric hybridizations. Bang *et al.* (1996 to 2009) and Jeong *et al.* (2003 to 2009) inspected the post-fertilization barriers between cultivated species and wild relatives in intergeneric hybridizations, and produced many potential hybrid progeny through *in vitro* procedures for each of the five breeding strategies shown in Fig. 1.

2. Production of novel F_1 hybrid plants by means of embryo rescue

Until the 1980s, F_1 hybrid plants had been produced from interspecific and intergeneric hybridizations mainly among *B. rapa*, *B. nigra*, *B. oleracea*, *B. napus*, *B. juncea*, *B. carinata* and *R. sativus*. Subsequently, a number of F_1 hybrid plants were produced from interspecific and intergeneric hybridizations between *Brassica* crops and wild relatives in the genera *Brassica*, *Sinapis*, *Diplotaxis*, *Moricandia*, *Eruca*, and *Orychophragmus*. At least 45 species of wild relatives were reported in reviews of the literature on the production of F_1 hybrids (Harberd and McArthur 1980, Kaneko *et al.* 2009, Prakash *et al.* 1999). Numerous novel F_1 hybrids have been produced through the development of various embryo rescue techniques: embryo culture (Nishi *et al.* 1962, 1970), ovary culture (Inomata 1977, 1978a, 1978b, Matsuzawa 1983), ovule culture (Momotaz *et al.* 1998) and placenta culture (Bang *et al.* 2007), as well as through combinations of these techniques, namely ovary culture followed by embryo or ovule culture, placenta culture followed by embryo culture, and successive ovary, ovule, and embryo culture. Ovary culture has been widely performed to overcome abortion of hybrid embryos during early stages of development, but this technique failed when a species such as *B. oleracea* with high regeneration ability was used as the pistillate parent. When *R. sativus* and *B. oleracea* are used as the pistillate parent, embryo culture is effective, although the hybrid embryo must develop to at least the heart-shaped stage before culture can begin. Placenta culture, in which placenta-attached ovules are removed and cultured starting about 15 days after pollination, may be more effective than ovule and embryo culture when *B. oleracea* is used as the pistillate parent.

3. Synthetic amphiploid lines

Synthetic amphidiploid lines have been developed by doubling the chromosome number of F_1 hybrid plants as well as by means of somatic cell fusion. Researchers have sporadically obtained amphidiploid plants in the F_2 generation of amphihaploid F_1 hybrids through fusion of the unreduced F_1 female and male gametes. These amphidiploid lines show low pollen and seed fertility due to unequal chromosome segregation in pollen mother cells in initial generations. With advancing generations, however, the hybrids generally develop high pollen and seed fertility as a result of increasing meiotic stabilization (Howard 1938, Kato and Tokumasu 1976, Sarashima 1973). Six artificial amphidiploid *Brassica* lines, four *Raphanobrassica* lines and three *Brassicoraphanus* lines have been developed and maintained for use as breeding materials (Table 1 and Fig. 2).

Synthetic fertile amphidiploid lines derived from hybrids between cultivated crops and their wild relatives have been used as genetic stocks in breeding programs, and as bridging materials for the transfer of desirable traits from wild species into cultivated ones. The fertile amphidiploid lines obtained through doubling of the chromosome number of sterile hybrids or through somatic hybridization between a cultivated crop and wild relatives have led to novel crops, including 'Hakuran' (*Brassica campestris* × *B. oleracea*), 'Radicole' (*Raphanus sativus* × *B. oleracea*), and 'Raparadish' (*B. campestris* × *R. sativus*) (Namai 1987). As a result, several novel cultivars, 'Gifu-Green' (Takada 1985), 'Senpousai No. 1 and No. 2' (Nagano 1988), 'Hanakkori'

Table 1. Self-pollinated seed fertility in artificial amphidipoids from reciprocal crosses among monogenomic *Brassica* crops as well as *Raphanus sativus*

Cross combination	Genome	Generations	No. of individuals observed	Seed fertility
B. rapa × *B. oleracea*	AACC	F_4	6	0.72 ± 0.63
B. oleracea × *B. rapa*	CCAA	F_4	10	1.19 ± 2.11
B. rapa × *B. nigra*	AABB	F_7	1	7.51
B. nigra × *B. rapa*	BBAA	F_6	3	2.21 ± 1.19
B. nigra × *B. oleracea*	BBCC	F_6	4	9.16 ± 2.48
B. oleracea × *B.nigra*	CCBB	F_7	5	1.55 ± 0.99
R. sativus × *B. oleracea*	RRCC	F_{10}	3	2.39 ± 0.88
B. oleracea × *R. sativus*	CCRR	F_4	5	0.76 ± 0.46
R. sativus × *B. rapa*	RRAA	F_7	–	1.3[a]
B.rapa × *R. sativus*	AARR	F_7	–	0.01[b]

B. napus 'N350' is 21.15seeds, *B. juncea* 'Aso-Takana' 11.54, and *B. carinata*.
Ca115' 8.72, respectively.
[a] Data from Akaba *et al.* 2009b.
[b] Data from Matsuzawa *et al.* 2000.

Fig. 2. Artificial synthetic amphidiploid plants. (A) *Raphanobrassica* (2n = 36, RRCC) derived from hybrid between *R. sativus* (2n = 18, RR) and *B. oleracea* (2n = 18, CC), (B) *Brassicoraphanus* (2n = 34, FFRR) derived from hybrid between *B. maurorum* (2n = 16, FF) and *R. sativus* (2n = 18, RR), (C) *Brassicoraphanus* (2n = 50, FFFFRR) derived from hybrid between *B. fruticulosa* (2n = 32, FFFF) and *R. sativus* (2n = 18, RR), (D) *Brassicoraphanus* (2n = 36, OORR) derived from hybrid between *B. oxyrrhina* (2n = 18, OO) and *R. sativus* (2n = 18, RR).

(Matsumoto *et al.* 1997), and 'F$_1$-Hosoda-Wase' (Takada 2006), were developed from amphidiploids between *B. rapa* and *B. oleracea*, and have been released by Japanese seed companies. Recently, we produced a number of amphidiploid lines between crop cultivars and wild relatives: *Brassica maurorum* × *R. sativus* (Bang *et al.* 1997, 1998, Fig. 2B), *Brassica fruticulosa* × *R. sativus* (Bang *et al.* 1997, 2000, Fig. 2C), *Brassica oxyrrhina* × *R. sativus* (Bang *et al.* 1997, Matsuzawa *et al.* 1997, Fig. 2D), *B. oxyrrhina* × *B. rapa* (Bang, unpublished data), *B. oxyrrhina* × *B. oleracea* (Bang, unpublished), *B. rapa* × *Diplotaxis tenuifolia* (Jeong *et al.* 2009), and *B. oleracea* × *D. tenuifolia* (Bang, unpublished). *Diplotaxis tenuifolia* has considerable potential as a healthy leafy salad vegetable because of its content of bioactive photochemicals, such as those that are involved in intermediate C$_3$–C$_4$ photosynthetic activity, and is commonly eaten as "rocket", a name it shares with some *Eruca* species (Martínez-Sánchez *et al.* 2007). The amphidiploid line of *B. rapa* × *D. tenuifolia* has desirable morphological characteristics, vigorous growth, and the attractive fragrance of *D. tenuifolia*, but its seed fertility was insufficient for commercial production as a crop. However, the line produced abundant seeds that grew into sesquidiploid plants, which inherited desirable traits when they were backcrossed with commercial cultivars of *B. rapa* (Jeong *et al.* 2009). Therefore, the amphidiploid lines of *B. rapa* × *D. tenuifolia* and *B. oleracea* × *D. tenuifolia* could both be useful genetic resources for the breeding of new leafy salad vegetables.

4. Monosomic alien chromosome addition lines

Chromosome addition lines, such as monosomic alien chromosome addition lines (MAALs), have been examined for genetic analysis in breeding programs for agronomic traits, and in studies of genes that are assumed to be located on the added chromosome. The advantages of using MAALs involve the possibility of assigning species-specific genes or characteristics to particular chromosomes, and the potential to transfer desirable agronomic traits between species (Matsuzawa *et al.* 1996, McGrath and Quiros 1990, Namai 1987, Prakash and Chopra 1990). Since Kaneko *et al.* (1987) produced MAALs that combined *R. sativus* with *B. oleracea* and Quiros *et al.* (1987) produced MAALs that combined *B. rapa* with *B. oleracea*, several MAALs have been bred through interspecific and intergeneric hybridizations between crop species and wild relatives. These include combinations of *B. campestris* with *Brassica alboglabra* (Chen *et al.* 1992), *R. sativus* with *E. sativa* (Bang 1996), *R. sativus* with *Sinapis arvensis* (Bang 1996), *B. campestris* with *B. oxyrrhina* (Srinivasan *et al.* 1998), *R. sativus* with *Moricandia arvensis* (Bang *et al.* 2002), *B. napus* with *Sinapis alba* (Wang *et al.* 2005), *B. napus* with *R. sativus* (Akaba *et al.* 2009b), and *B. napus* with *B. juncea* (Takashima *et al.* 2012).

To produce and classify MAALs, the cross-incompatibility that exists in successive backcrosses with the background species (as the recurrent parent) must be overcome, and it is essential to use specific markers for the chromosome from the donor species to assist in selection. The cross-incompatibility that often appears in successive backcrosses of the amphidiploid (F$_1$) progeny with the background species seems to depend upon the genetic affinity between the background species and the chromosome donor. These barriers in the first backcross may be reflected in male and female sterility of the amphidiploid as well as in pre- and post-fertilization barriers (Table 2). In the intergeneric hybridization between *M. arvensis* and *R. sativus* (Bang *et al.* 1996a), the amphidiploid plants showed more stable chromosome association at metaphase I in pollen mother cells

Table 2. Seed fertility by backcrossing between amphidiploid plants and their parents

Pistillate parent	Genome	Male parent	Genome	Seed fertility
Artificial *B. napus*	AACC	*B. rapa*	AA	2.50 ± 1.55
Natural *B. napus*	AACC	ditto		9.47 ± 3.42
Artificial *B. napus*	CCAA	ditto		3.06 ± 3.13
Artificial *B. juncea*	AABB	*B. rapa*	AA	0.26
Natural *B. juncea*	AABB	ditto		9.01 ± 3.40
Artificial *B. juncea*	BBAA	ditto		2.25 ± 0.62
Artificial *B. carinata*	BBCC	*B. oleracea*	CC	1.72 ± 0.58
Natural *B. carinata*	BBCC	ditto		0.37 ± 0.04
Artificial *B. carinata*	CCBB	ditto		0.01 ± 0.01
Raphanobrassica	RRCC	*R. sativus*	RR	0.12 ± 0.06[a]
Raphanobrassica	RRAA	*R. sativus*	RR	0.03 ± 0.03
Brassicoraphanus	AARR	*B. rapa*	AA	0.95 ± 1.59

[a] Data from Kaneko *et al.* (1987).

and higher pollen fertility than the amphihaploid plants. In the backcross of the amphidiploid with the parent *R. sativus*, there was no pre-fertilization barrier, but a post-fertilization barrier appeared in the form of abortion of the hybrid embryos, although this was overcome using an embryo rescue technique. Occasionally, male and female sterility of the BC_1 hybrid plants (sesquidiploids) and subsequent pre- and post-fertilization barriers also appear in successive back-crossing (usually by BC_2). On the other hand, these barriers in the first and second backcrosses were not observed in other cross-combinations to *R. sativus* × *S. arvensis* (Bang 1996), *R. sativus* × *E. sativa* (Bang 1996), and *B. campestris* × *B. oxyrrhina* (Srinivasan *et al.* 1998). However, whole MAALs have not been developed from any cross-combinations in the Brassicaceae thus far. The genetic system that controls hybridization barriers in interspecific and intergeneric hybridizations seems to function between the nuclear and cytoplasmic genomes in the recurrent parent's genetic background and a few chromosomes from the donor genome.

These MAALs have been effectively exploited to ana-lyze various agronomic and genetic traits to homoeologous relationships in the genus *Brassica* (Kaneko *et al.* 2002); disease resistance to blackleg (*Leptosphaeria maculans*; Chevre *et al.* 1996), turnip mosaic virus (TuMV; Kaneko *et al.* 1996), beet cyst nematode (*Heterodera schachtii*; Peterka *et al.* 2004), and clubroot (*Plasmodiophora brassicae*; Akaba *et al.* 2009a); and photorespiratory characteristics (Bang *et al.* 2009); and in restoring pollen fertility (Akaba *et al.* 2009b, Budahn *et al.* 2008) and avoiding lethality in an alloplasmic line (Tsutsui *et al.* 2011).

5. Alloplasmic lines

The production of F_1 hybrid seed of *Brassica* crops is cur-rently based on either self-incompatibility or cytoplasmic male sterility (CMS). The self-incompatibility controlled by *S* alleles is not sufficiently stable because it is affected by various environmental factors (Horisaki and Niikura 2004). CMS is a maternally inherited trait, and has been ascer-tained to be more successful because of the stable expres-sion of pollen sterility without any obvious changes in vege-tative growth or female fertility. Alloplasmic lines have been developed by exchanging the cytoplasm through inter-specific and intergeneric hybridizations followed by succes-sive backcrossing to the nuclear genome's donor species. These hybrids acquire the advantages of CMS and useful agronomic traits, such as herbicide resistance, modification of flavor and nutrient content, and morphogenetic potential (Namai 1987).

Sarashima *et al.* (1990a, 1990b, 1990c) produced many kinds of alloplasmic lines of monogenomic Brassicaceae crops (*B. rapa*, *B. oleracea*, and *R. sativus*), but most of them did not exhibit any agronomically useful traits, except for CMS of *B. oleracea*, which carried *R. sativus* cytoplasm but no *R. sativus* nuclear genetic material. The CMS in the latter alloplasmic line may have been induced by a lack of genetic affinity between the mitochondrial genome and the nuclear genome. The cytoplasm from several wild relatives has been introduced as a source of CMS into several *Brassica* crops (*B. rapa* [*B. campestris*], *B. juncea*, and *B. napus*) through interspecific and intergeneric hybridiza-tions followed by successive backcrossings. These wild rel-atives include *Arabidopsis thaliana*, *Diplotaxis berthautii*, *Diplotaxis muralis*, *Diplotaxis catholica*, *Diplotaxis siifolia*, *Diplotaxis erucoides*, *B. oxyrrhina*, *Brassica tournefortii*, *Enarthrocarpus lyratus*, *E. sativa*, *Eruca vesicaria*, *M. arvensis*, and *Trachystoma ballii* (reviewed by Kaneko *et al.* 2009, Prakash *et al.* 1999). Recently, some new CMS sources have been introduced into several *Brassica* crops in addition to *R. sativus*, including *B. maurorum* (Bang *et al.* 2011), *B. oxyrrhina* (Shim *et al.* 2010, 2011a, 2012), *D. tenuifolia* (Shim *et al.* 2011b), and *B. fruticulosa* (Tsutsui 2013).

When alloplasmic lines are produced through interspecif-ic and intergeneric hybridizations followed by successive backcrossings, there are various affinity natures that can be observed in cross-combinations and in different genera-tions. For example, two alloplasmic *B. juncea* lines were produced using amphidiploid lines of *D. erucoides* × *B. campestris* and *D. berthautii* × *B. campestris* as cytoplas-mic donor plants (Malik *et al.* 1999). Alloplasmic *B. napus* carrying *A. thaliana* cytoplasm was obtained from six back-crosses of the somatic hybrid to *B. napus* (Leino *et al.* 2003). Alloplasmic *B. campestris* with *E. sativa* cytoplasm was produced via a sesquidiploid plant obtained from an amphihaploid F_1 hybrid by open pollination (Matsuzawa *et al.* 1996), and alloplasmic *R. sativus* with *E. vesicaria* cyto-plasm was produced via the same method (Bang 1996). Al-loplasmic *R. sativus* with *M. arvensis* cytoplasm was also produced via a sesquidiploid plant generated from back-crossing of an amphidiploid F_1 hybrid to *R. sativus*, fol-lowed by embryo rescue (Bang *et al.* 1996a, 2002). As Namai (1976) indicated, sesquidiploid plants can be useful as a bridge plant to generate an alloplasmic line with no fer-tilization barriers in various interspecific and intergeneric hybridizations.

Occasionally, male or female sterility of sesquidiploid plants and subsequent pre- and post-fertilization barriers also appear in successive backcrossings. When this hap-pens, an alloplasmic line cannot be generated. The pre- and post-fertilization barriers can often be overcome by using bud-pollination and embryo rescue, respectively. The fe-male sterility of sesquidiploid plants, which is a primary cause of hybrid breakdown, can also be overcome by using F_1 cultivars as the pollen parents and subsequently as the recurrent parent because male and female gametes with di-verse genotypes can be confirmed in each generation. In sesquidiploid hybrids between *Brassica* crop species and their wild relatives, Tsutsui *et al.* (2011) found that many progeny involving alloplasmic lines could be generated through repeated pollination without embryo rescue, but

that no progeny could be obtained by bud pollination and subsequent embryo rescue techniques owing to severe chlorosis. It is likely that the female sterility in the sesquidiploid plants was caused by delayed maturation of the egg cells. As mentioned above, using synthetic allopolyploids between different combinations as bridge plants to transfer cytoplasm from wild relative species and ascertaining the cause of sexual incompatibility barriers in the hybrid plants as accurately as possible may provide practical ways to improve the efficiency of producing alloplasmic lines.

6. Alien gene introgression lines

Until the 1980s, alien gene introgression lines had been produced by hybridization mainly between the Brassicaceae crops, resulting in the development of novel cultivars with desirable agronomic traits. Examples include Chinese cabbage (*B. rapa*) cultivars resistant to bacterial soft rot (Shimizu *et al.* 1962), early-maturing cultivars of *B. napus* (Namai 1976, 1987), and virus-resistant cultivars of *B. oleracea* (Namai 1976). Warwick (1993) described wild genera of the tribe Brassiceae as the sources of various agronomically interesting traits, such as hairiness, resistance to pod shattering, photosynthesis, soil adaptation and disease resistance. A large collection of wild relative species of *Brassica* crops have been screened as sources of resistance to blackleg (also called stem canker), alternaria leaf spot (*Alternaria* spp.), and clubroot. Interspecific and intergeneric hybrids between wild relatives and *Brassica* crop species, and their progeny, would certainly provide valuable breeding materials for the introgression of agronomically useful traits. Siemens (2002) reviewed the possibility of using interspecific and intergeneric hybridizations between *B. napus* and some wild relatives in the tribe Brassiceae to introduce resistance to important fungal pathogens. As Namai (1987) indicated, the introgression of desirable genes from one species to another in the Brassiceae has been accomplished through meiotic allosyndesis in *Brassica* crops. Bang (1996) described the homoeologous pairing between an *R. sativus* chromosome and an *S. arvensis* chromosome in an *S. arvensis* monosomic addition line of *R. sativus* (MAAL-d type). A pollen fertility restoration gene from the cytoplasmic donor species has been transferred to alloplasmic *Brassica* oilseed crops through homoeologous pairing between the AB and M chromosomes in an *M. arvensis* MAAL of *B. juncea* (Prakash *et al.* 1999), and between the A and E chromosomes in an *E. lyratus* MAAL of *B. rapa* (Banga *et al.* 2003). Recently, resistance to the beet cyst nematode and clubroot has been transferred from *R. sativus* to *B. napus* using *R. sativus* MAALs of *B. napus* (Akaba *et al.* 2009a, Budahn *et al.* 2008). Analysis of genes and genomes and the efficient induction and identification of recombination for introgression might be prerequisites for the development of an alien gene introgression line. The genetic linkage maps that have been recently constructed for some *Brassica* crops will offer useful tools to support genetic introgression.

7. Monosomic alien chromosome substitution lines

Many disomic alien chromosome substitution lines (DASLs) have been bred in wheat cultivars. In these lines, one member of a chromosome pair is replaced by a homoeologous chromosome from *Secale*, *Hordeum*, and *Aegilops* (Khush 1973). Such DASLs can be used to study the genetic effects of individual chromosomes, and to estimate the number of genes that control a given trait and their linkage relationships. These lines can be generally developed in the progeny after self-fertilization of a monosomic alien chromosome substitution line (MASL). In this breeding system, the MASLs can be generated only when the two corresponding chromosomes are homoeologous and complement each other, especially within diploid species. In the Brassicaceae, DASLs and MASLs generated using the technique described above have not been found, as far as we know. DASLs are known to occur spontaneously during successive backcrossings after interspecific hybridization. By analyzing the meiotic configuration, Banga (1988) reported the development of C genome chromosome substitution lines in *B. juncea* that arose spontaneously in an interspecific hybridization between *B. juncea* and *B. napus*. Although phylogenetic relationships have been suggested in *Brassica* (Armstrong and Keller 1981, 1982, Röbbelen 1960) as well as between *Brassica* and allied genera (Quiros *et al.* 1988), we cannot currently confirm the generation of MASLs among segregated amphiploid lines, as techniques are not currently available for the critical analysis and identification of each chromosome. Chen *et al.* (1997) produced *B. alboglabra* chromosome substitution *B. rapa* lines. In this context, *in situ* hybridization procedures, the production of somatic chromosome maps by various imaging methods, and the development of species-specific markers would strongly support the development and analysis of MASLs.

Conclusions

Five categories of hybrid progeny can be produced through interspecific and intergeneric hybridization: synthetic amphidiploid lines, alien gene introgression lines, alloplasmic lines, monosomic alien chromosome addition lines, and monosomic alien chromosome substitution lines. These approaches can provide materials that are useful both for developing practical breeding strategies and for studying the genetic effects of individual chromosomes on plant traits. This includes estimating the number of genes that control a trait, describing their linkage relationships, and using this knowledge for genetic improvement of Brassicaceae crops. For these research programs to be successful, it will be necessary to produce and maintain as many true lines as possible of these five hybrid types.

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